



## Under The Microscope

### Spotlight on Laboratory Issues

## The ISDH Mycobacteriology Laboratory

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Among infectious diseases, tuberculosis (TB) remains the second leading killer of adults in the world, with more than 2 million TB-related deaths each year. An estimated 2 billion persons are infected with *Mycobacterium tuberculosis*, the bacterium that causes tuberculosis. This year, there will be more than 8 million new cases of TB reported worldwide.

While the tuberculosis data for the United States paints a much brighter picture than that of many other countries, recent trends are cause for concern. Despite a decline in tuberculosis cases nationwide, rates have increased in certain states, including Indiana, and elevated TB rates continue to be reported in certain populations (e.g., foreign-born persons and racial/ethnic minorities). One important aspect of reversing these trends is rapid identification of the bacteria by laboratories.

The Indiana State Department of Health (ISDH) Mycobacteriology Laboratory is dedicated to protecting the citizens of Indiana through accurate and timely testing of TB isolates. The laboratory is staffed by three microbiologists and one laboratory technician with almost 50 years of combined experience in mycobacteriology. The TB lab utilizes state-of-the-art technology, as well as traditional culture methods to identify mycobacteria in submitted specimens and reference cultures.

In 2003, the TB laboratory received and processed over 2,700 specimens, with over 12% testing positive for TB. Nearly 170 specimens (6%) tested positive for mycobacteria species other than tuberculosis (MOTT). The lab also received 365 reference cultures in 2003. Over 20% of the cultures were positive for TB, while MOTT was identified for more than 75%.



Laboratory technician, Laura Taube, setting up to process TB specimens; photo courtesy of Mark

Specimens received in the TB laboratory are logged in and processed each morning. Processing involves a decontamination step to rid the specimen of normal flora bacteria and a concentration step to increase the likelihood of growth in media. Both liquid and solid media are inoculated from the processed specimen. A smear is also prepared to check the specimen for the presence of acid-fast bacilli (AFB). The slides are read daily utilizing fluorescent microscopy, with positive results faxed to the submitter the same day.

Primary identifications of suspected growth from liquid and solid media are done by High Performance Liquid Chromatography (HPLC). Isolates are processed with a multi-step procedure involving the extraction of the mycolic acids from the cell wall of the bacteria and the derivatization of the acids. Samples are then run through the HPLC instrument, producing distinct chromatographic patterns. Each species of mycobacteria has a unique pattern, which aids the experienced microbiologist in making a definitive identification.

The TB lab also utilizes species-specific DNA probes as needed for identification purposes (<1% of identifications).

Another vital service provided by the TB laboratory is antibiotic susceptibility testing of all new TB cases. The lab performs susceptibility tests for the five primary TB drugs: isoniazid (INH), rifampin, ethambutol, streptomycin, and pyrazinamide (PZA). The laboratory also repeats drug sensitivities for patients who remain culture positive after three months of therapy. Accurate susceptibility testing is an important part of the TB control program that helps to minimize the number of multi-drug resistant TB cases.

Earlier this year, the TB laboratory began participating in the Centers for Disease Control and Prevention (CDC) TB Genotyping Program. The lab submits an isolate from every culture-positive patient with TB to a genotyping laboratory in Michigan under contract with CDC. TB genotyping identifies genetic links between *Mycobacterium tuberculosis* isolates from different TB patients. TB genotyping results, when combined with epidemiologic data, will help outbreaks to be detected earlier and controlled more rapidly, as well as identify incorrect TB diagnoses based on false-positive culture results more easily.

The ISDH Mycobacteriology Laboratory continues to investigate the latest technologies for TB testing to ensure the timeliest and most accurate results possible. As an integral part of Indiana's TB Control Program, the TB lab is striving for the ultimate goal of the elimination of tuberculosis in the United States.

## References:

1. CDC. *Trends in Tuberculosis-United States, 1998-2003*. MMWR 2004; 53.
  2. National TB Controllers Association. *Guide to the Application of Genotyping to Tuberculosis Prevention and Control*. U.S Department of Health and Human Services, CDC; June 2004.
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